CLAIMS

1. A modified thermostable DNA polymerase wherein in the $DX_1EX_2X_3X_4H$ sequence (D: aspartic acid, E: glutamic acid, H: histidine, X_1, X_2, X_3 and X_4: any amino acid) consisting of DX_1E sequence within the EXO I region and a four amino acid length peptide adjacent to said glutamic acid(E) of thermostable DNA polymerase having 3'-5' exonuclease activity, histidine(H) has been replaced by another amino acid.

2. The modified thermostable DNA polymerase according to claim 1, wherein in the $DX_1EX_2X_3X_4H$ sequence, histidine(H) has been replaced by an amino acid selected from the group consisting of aspartic acid, glutamic acid, tyrosine, alanine, lysine and arginine.

3. The modified thermostable DNA polymerase according to claim 1 having the following physicochemical properties:

(1) DNA extension rate: at least 20 bases/second; and

(2) thermostability: it is capable of retaining 10% or more DNA polymerase activity of untreated DNA polymerase at pH 8.8 (determined at 25°C) after treatment at 95°C for 6 hours.

4. The modified thermostable DNA polymerase according to claim 3 having the following physicochemical

properties:

(1) DNA extension rate: at least 30 bases/second;

(2) thermostability: it is capable of retaining 40% or more DNA polymerase activity of untreated DNA polymerase at pH 8.8 (determined at 25°C) after treatment at 95°C for 6 hours; and

(3) amino acid sequence: in the DIETLYH sequence (D: aspartic acid, I: isoleucine, E: glutamic acid, T: threonine, L: leucine, Y: tyrosine, H: histidine) located at the 141- to 147-positions in the amino acid sequence of SEQ ID NO: 2, histidine(H) has been replaced by another amino acid.

5. The modified thermostable DNA polymerase according to claim 4 having the following physicochemical properties:

(1) DNA extension rate: at least 30 bases/second;

(2) thermostability: it is capable of retaining 60% or more DNA polymerase activity of untreated DNA polymerase at pH 8.8 (determined at 25°C) after treatment at 95°C for 6 hours; and

(3) amino acid sequence: in the DIETLYH sequence (D: aspartic acid, I: isoleucine, E: glutamic acid, T: threonine, L: leucine, Y: tyrosine, H: histidine) located at the 141- to 147-positions in the amino acid sequence of SEQ ID NO: 2, histidine(H) has been replaced by another

amino acid.

6. The modified thermostable DNA polymerase according to claim 5, wherein in the amino acid sequence of SEQ ID NO: 2, histidine(H) at the 147-position has been replaced by an amino acid selected from the group consisting of aspartic acid, glutamic acid, tyrosine, alanine, lysine and arginine.

7. The modified thermostable DNA polymerase according to claim 6, wherein in the amino acid sequence of SEQ ID NO: 2, histidine(H) at the 147-position has been replaced by aspartic acid.

8. The modified thermostable DNA polymerase according to claim 6, wherein in the amino acid sequence of SEQ ID NO: 2, histidine(H) at the 147-position has been replaced by glutamic acid.

9. The modified thermostable DNA polymerase according to claim 6, wherein in the amino acid sequence of SEQ ID NO: 2, histidine(H) at the 147-position has been replaced by tyrosine.

10. The modified thermostable DNA polymerase according to claim 6, wherein in the amino acid sequence of SEQ ID NO: 2, histidine(H) at the 147-position has been replaced by alanine.

11. The modified thermostable DNA polymerase according to claim 6, wherein in the amino acid sequence

of SEQ ID NO: 2, histidine(H) at the 147-position has been replaced by lysine.

12. The modified thermostable DNA polymerase according to claim 6, wherein in the amino acid sequence of SEQ ID NO: 2, histidine(H) at the 147-position has been replaced by arginine.

13. A gene encoding a modified thermostable DNA polymerase wherein in the $DX_1EX_2X_3X_4H$ sequence (D: aspartic acid, E: glutamic acid, H: histidine, X_1, X_2, X_3 and X_4: any amino acid) consisting of DX_1E sequence within the EXO I region and four amino acid length peptide adjacent to said glutamic acid(E) of thermostable DNA polymerase having 3'-5' exonuclease activity, histidine(H) has been replaced by another amino acid.

14. The gene according to claim 13 which encodes a modified thermostable DNA polymerase having the following physicochemical properties:

(1) DNA extension rate: at least 20 bases/second; and

(2) thermostability: it is capable of retaining 10% or more DNA polymerase activity of untreated DNA polymerase at pH 8.8 (determined at 25°C) after treatment at 95°C for 6 hours.

15. The gene according to claim 13 which encodes a modified thermostable DNA polymerase having the following physicochemical properties:

(1) DNA extension rate: at least 30 bases/second;

(2) thermostability: it is capable of retaining 40% or more DNA polymerase activity of untreated DNA polymerase at pH 8.8 (determined at 25°C) after treatment at 95°C for 6 hours; and

(3) amino acid sequence: in the DIETLYH sequence (D: aspartic acid, I: isoleucine, E: glutamic acid, T: threonine, L: leucine, Y: tyrosine, H: histidine) located at the 141- to 147-positions in the amino acid sequence of SEQ ID NO: 2, histidine(H) has been replaced by another amino acid.

16. The gene according to claim 13 which encodes a modified thermostable DNA polymerase having the following physicochemical properties:

(1) DNA extension rate: at least 30 bases/second;

(2) thermostability: it is capable of retaining 60% or more DNA polymerase activity of untreated DNA polymerase at pH 8.8 (determined at 25°C) after treatment at 95°C for 6 hours; and

(3) amino acid sequence: in the DIETLYH sequence (D: aspartic acid, I: isoleucine, E: glutamic acid, T: threonine, L: leucine, Y: tyrosine, H: histidine) located at the 141- to 147-positions in the amino acid sequence of SEQ ID NO: 2, histidine(H) has been replaced by another amino acid.

17. A recombinant DNA vector obtained by inserting the gene of any one of claims 13 to 16 into an expression vector.

18. The recombinant DNA vector according to claim 17, wherein the expression vector is pLED-MI, pBluescript or their derivatives.

19. A transformant produced by transforming a host cell with the recombinant DNA vector of claim 17 or 18.

20. The transformant according to claim 19 wherein the host cell is Escherichia coli.

21. A process for producing a modified thermostable DNA polymerase, which comprises culturing the transformant of claim 20 and recovering the thermostable DNA polymerase from the culture broth.

22. A method for amplifying or extending nucleic acid, which comprises reacting DNA as a template, one or more kinds of primers, dNTP and the thermostable DNA polymerase of any one of claims 1 to 12, thus extending the primer(s) to synthesize DNA primer extension product(s).

23. The method for amplifying nucleic acid according to claim 22, wherein the primers are 2 kinds of oligonucleotides, each of the primers being complementary to a DNA extension product of the other primer.

24. The method for amplifying nucleic acid according to claim 23, which comprises heating and cooling repeatedly.

25. A reagent kit for amplifying nucleic acid, which comprises 2 kinds of primers, each of the primers being complementary to a DNA extension product of the other primer; dNTP; the thermostable DNA polymerase of any one of claims 1-12; divalent ion(s); monovalent ion(s); and a buffer solution.

26. A reagent kit for amplifying nucleic acid, which comprises 2 kinds of primers, each of the primers being complementary to a DNA extension product of the other primer; dNTP; the thermostable DNA polymerase of any one of claims 1-12; magnesium ion; at least one of monovalent ions selected from the group consisting of ammonium ion and potassium ion; BSA (bovine serum albumin); a nonionic surfactant and a buffer solution.

27. A reagent kit for amplifying nucleic acid, which comprises 2 kinds of primers, each of the primers being complementary to a DNA extension product of the other primer; dNTP; the thermostable DNA polymerase of any one of claims 1-12; magnesium ion; at least one of monovalent ions selected from the group consisting of ammonium ion and potassium ion; BSA (bovine serum albumin); a nonionic surfactant; a buffer solution and an

antibody capable of suppressing at least one activity selected from polymerase activity and 3'-5' exonuclease activity of the thermostable DNA polymerase.

28. A DNA polymerase composition which comprises one or more kinds of modified thermostable DNA polymerases defined in any of claims 1-12.

29. A method of producing a mutated DNA which comprises reacting DNA as a template, mutagenesis primers, dNTP and the thermostable DNA polymerase of any one of claims 1 to 12, thus extending the primers to synthesize DNA primer extension products.

30. A reagent kit for producing a mutated DNA which comprises mutagenesis primers, dNTP and the thermostable DNA polymerase of any one of claims 1 to 12.